

Figure 1

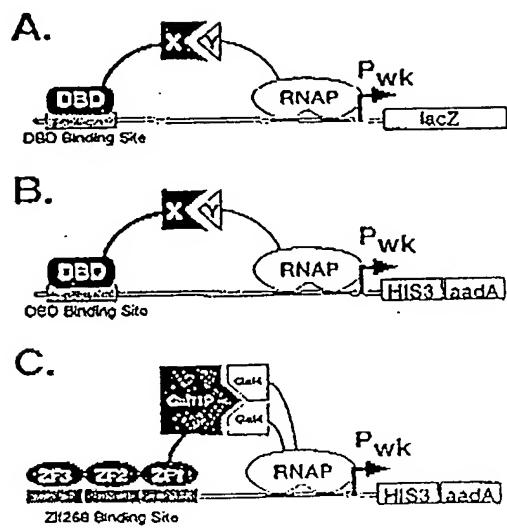


Figure 2

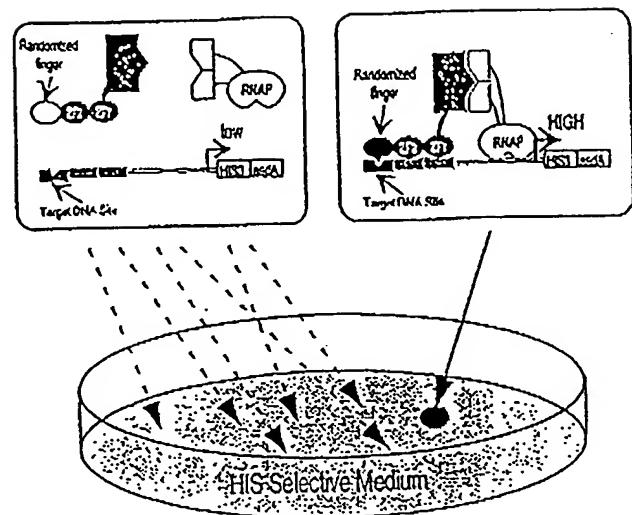


Figure 3

A. TATA Subsite Selection:

Phage Display Consensus: **3' AAA 5'**

-1	1	2	3	5	6
Q	+	-	N	--	

Group I:

Q R G N L V	N S G A Y N
Q K T N M V	N S G A W N
Q X Y N I L	N S G A F N
Q R Y N V V	N S G T H N
Q X G N K V	N T G A Y N
Q X G N R V	G S C A Y N
Q X G N M V	(4)
Q R G N K V	
Q R G N K T	
Q L G N M V	
Q K G N K V	
Q L G N K V	

Group II:

Q R G N K K (2)	Q K S N L R
Q R G N K K	Q X A N T K
Q R G N V K	Q K A N K L
Q X G N K K	Q L S N K X
Q X G N Y K	Q P G N K X (2)
Q K H N K K	Q K G N N V
Q K G N S R	Q K S N N V
Q S G N K K	Q K H N L R

B. p53 Subsite Selection:

Phage Display Consensus: **3' TGT 5'**

-1	1	2	3	5	6
H	-	H	H	--	

Group I:

H K H H K A (2)	
H L H H K A	

Group II:

Q R W L N R	W R S S L V*
Q R W L R R	W Q S S K V*
Q D N L N R	W Y S S I V*
Y K R D N R	F D S S R A*
A R W L N R	E R N R N R (4)
E R W R N R	E R W R N E
E R W R R R	E R W R I R
E R W R R R	E R W R S R
E R W R R R (2)	E R W R V R
E R W R R R	E R W R V R
E R W R R R	E R W R V R
E K W R R R	E R W R L R
E K W R R R (2)	E K W R T R
E G W R R R	E N W R K R
E R W R K R (2)	E Q W R K R
E R W R K R	E R W R M R

C. NRE Subsite Selection:

3' ACT 5'

-1	1	2	3	5	6
N S G S W K					

Group I:
first selection:
second selection:
phage display consensus:

N S G S W K (15)	
N S G S W K (2)	

T - T N - S

D. Group III Fingers:

-1	1	2	3	5	6
From the TATA Selection: R R W L K L	R K W L Q L				
R K W L K L	R K W L R L (2)				

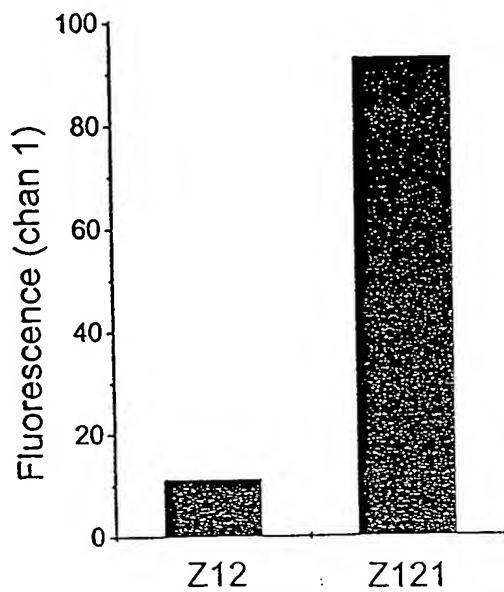
From the p53 Selection: R K W L R L	R K W L M L
R K W L R L	R K W I N L
R K W L R L	R K W Y Q L

From the NRE Selection: R K W L R L	R A W L K L
R K W L K L	R K W Y R L
R K W L K L	

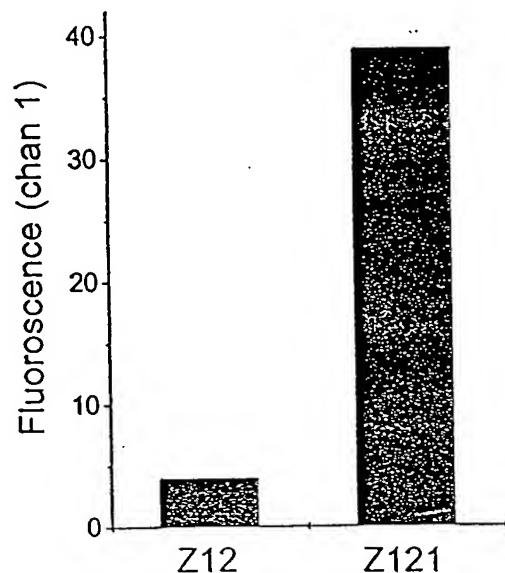
Behavior of different Fluorescent Proteins in the Bacterial Two-Hybrid System

Fig. 4

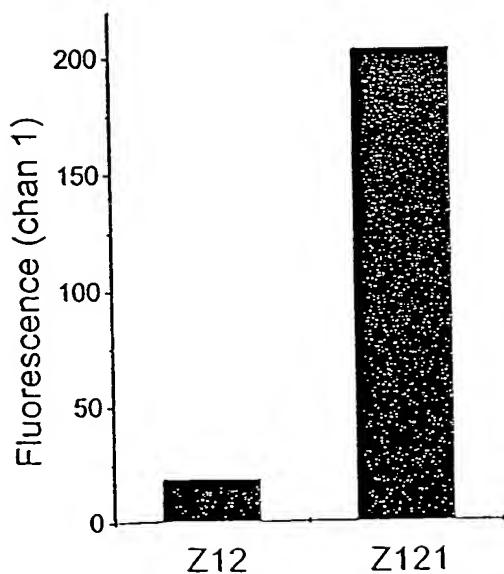
EGFP



GFP 3.1



EYFP



RFP (dsRED)

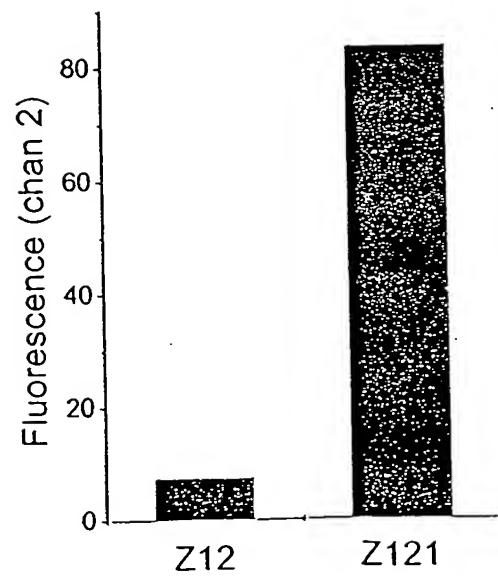


Figure 5.

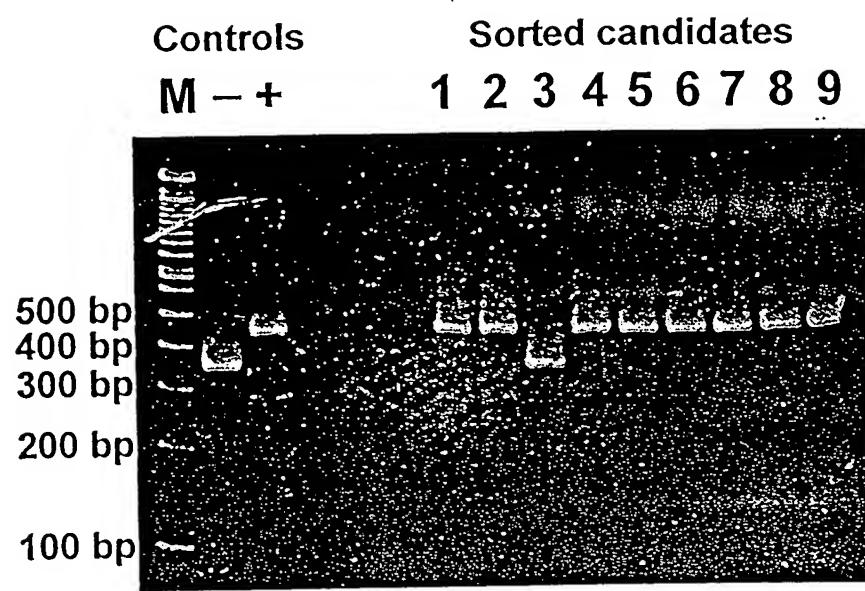


Figure 6

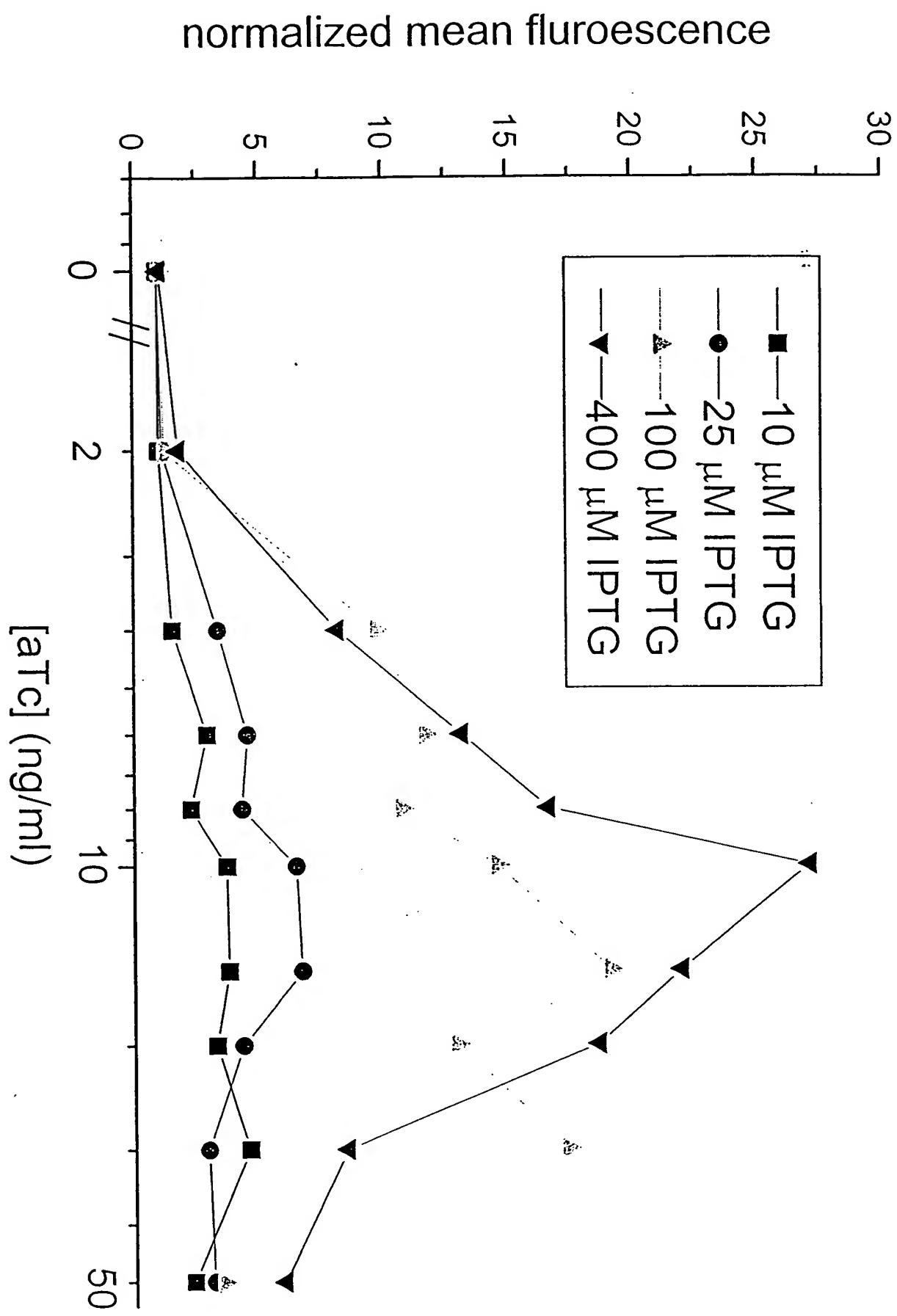


Figure 7

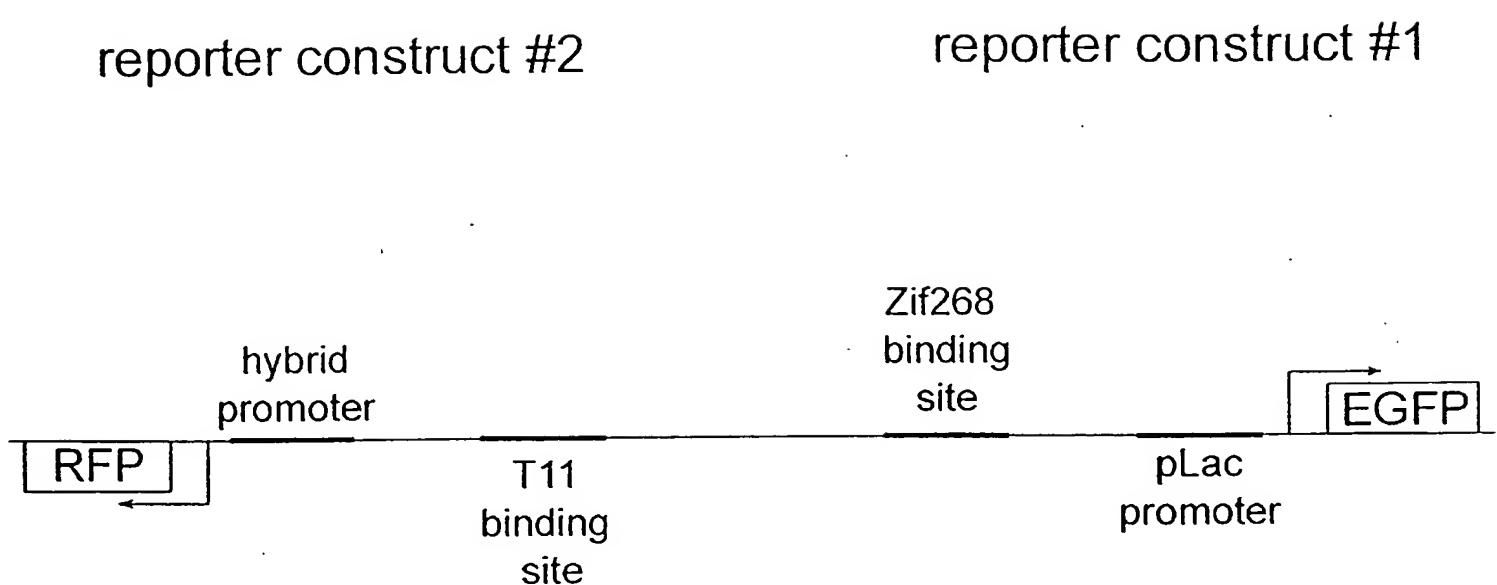
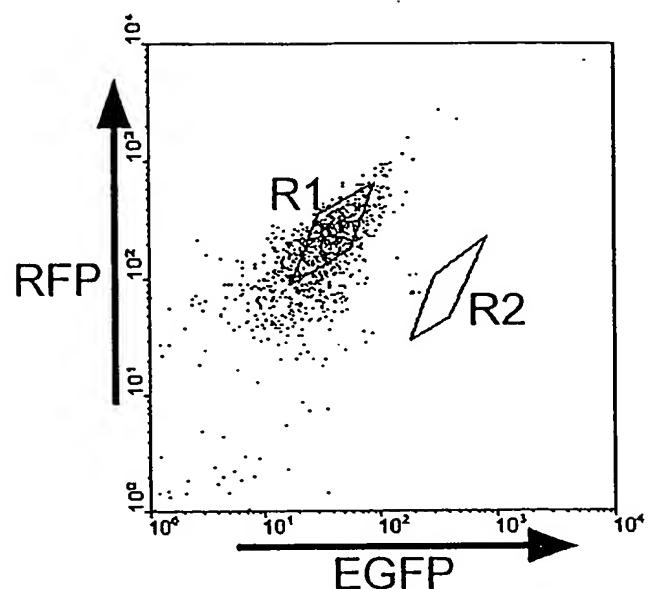
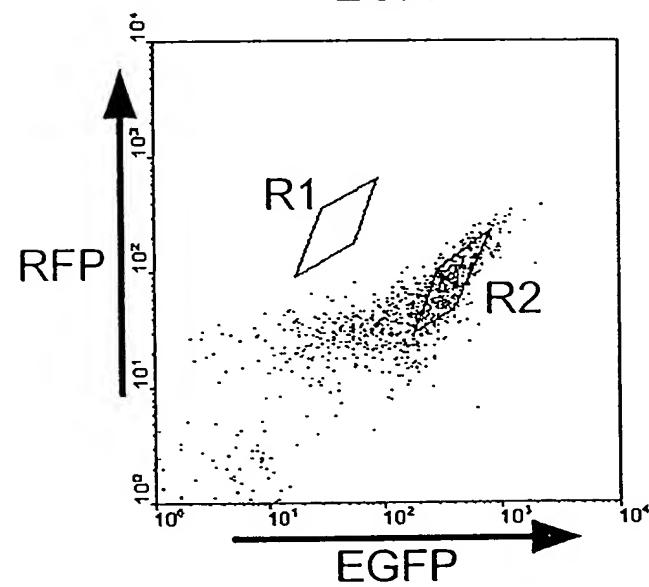


Figure 8

Two-Color reporter +
Gal11P-T11 protein



Two-Color reporter +
Gal11P-Zif268 protein



Two-Color reporter +
Gal11P-Z12 protein

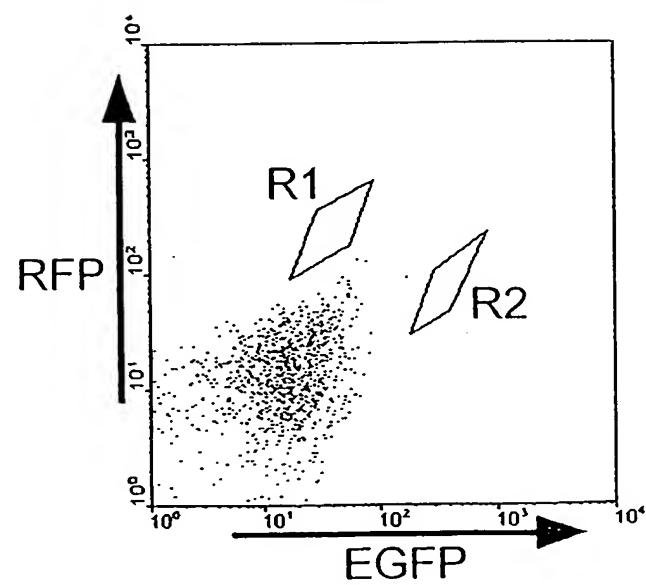


Fig 9

P53^{ZF} *in vitro* site selection consensus sequence:

CXGGACACGTX

(where X = no clear preference)

In vivo site selection library

CGGGANNNNNG

(where N = a mixture of A, G, C, and T)

Selected Clones:

Sequence	# of clones
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<u>CGGGACACGTG</u>	9
<u>CGGGACATGTG</u>	5
<u>CGGGACACGGG</u>	2

Sequence	Fold Activation
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<u>CGGGACACGTG</u>	18.6 ± 2.7
<u>CGGGACATGTG</u>	12.0 ± 0.5
<u>CGGGACACGGG</u>	12.6 ± 1.9